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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/31, C07K 14/315, 16/12, C12Q 1/68

A2

(11) International Publication Number: WO 98/18931

(43) International Publication Date: 7 May 1998 (07.05.98)

(21) International Application Number: PCT/US97/19588

(22) International Filing Date: 30 October 1997 (30.10.97)

(30) Priority Data:
60/029,960 31 October 1996 (31.10.96) US

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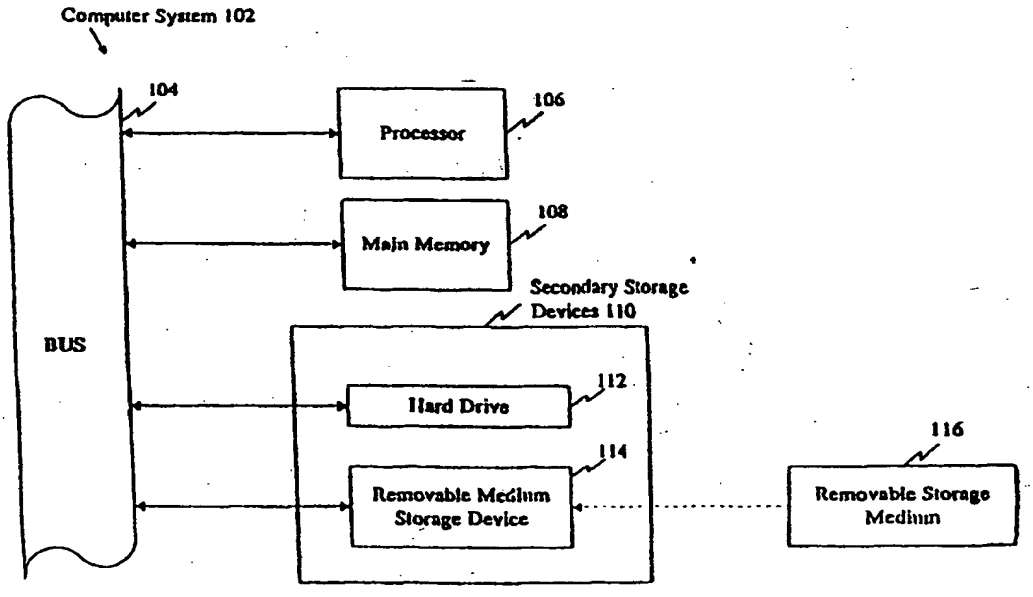
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: *STREPTOCOCCUS PNEUMONIAE* POLYNUCLEOTIDES AND SEQUENCES



(57) Abstract

The present invention provides polynucleotide sequences of the genome of *Streptococcus pneumoniae*, polypeptide sequences encoded by the polynucleotide sequences, corresponding polynucleotides and polypeptides, vectors and hosts comprising the polynucleotides, and assays and other uses thereof. The present invention further provides polynucleotide and polypeptide sequence information stored on computer readable media, and computer–based systems and methods which facilitate its use.

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presence of *Streptococcus pneumoniae* in a sample, hereinafter referred to as diagnostic fragments or DFs.

Each of the ORFs in fragments of the *Streptococcus pneumoniae* genome disclosed in Tables 1-3, and the EMFs found 5' to the ORFs, can be used in numerous ways as polynucleotide reagents. For instance, the sequences can be used as diagnostic probes or amplification primers for detecting or determining the presence of a specific microbe in a sample, to selectively control gene expression in a host and in the production of polypeptides, such as polypeptides encoded by ORFs of the present invention, particular those polypeptides that have a pharmacological activity.

The present invention further includes recombinant constructs comprising one or more fragments of the *Streptococcus pneumoniae* genome of the present invention. The recombinant constructs of the present invention comprise vectors, such as a plasmid or viral vector, into which a fragment of the *Streptococcus pneumoniae* has been inserted.

The present invention further provides host cells containing any of the isolated fragments of the *Streptococcus pneumoniae* genome of the present invention. The host cells can be a higher eukaryotic host cell, such as a mammalian cell, a lower eukaryotic cell, such as a yeast cell, or a procaryotic cell such as a bacterial cell.

The present invention is further directed to isolated polypeptides and proteins encoded by ORFs of the present invention. A variety of methods, well known to those of skill in the art, routinely may be utilized to obtain any of the polypeptides and proteins of the present invention. For instance, polypeptides and proteins of the present invention having relatively short, simple amino acid sequences readily can be synthesized using commercially available automated peptide synthesizers. Polypeptides and proteins of the present invention also may be purified from bacterial cells which naturally produce the protein. Yet another alternative is to purify polypeptide and proteins of the present invention from cells which have been altered to express them.

The invention further provides methods of obtaining homologs of the fragments of the *Streptococcus pneumoniae* genome of the present invention and homologs of the proteins encoded by the ORFs of the present invention. Specifically, by using the nucleotide and amino acid sequences disclosed herein as

a probe or as primers, and techniques such as PCR cloning and colony/plaque hybridization, one skilled in the art can obtain homologs.

The invention further provides antibodies which selectively bind polypeptides and proteins of the present invention. Such antibodies include both monoclonal and polyclonal antibodies.

The invention further provides hybridomas which produce the above-described antibodies. A hybridoma is an immortalized cell line which is capable of secreting a specific monoclonal antibody.

The present invention further provides methods of identifying test samples derived from cells which express one of the ORFs of the present invention, or a homolog thereof. Such methods comprise incubating a test sample with one or more of the antibodies of the present invention, or one or more of the DFs of the present invention, under conditions which allow a skilled artisan to determine if the sample contains the ORF or product produced therefrom.

In another embodiment of the present invention, kits are provided which contain the necessary reagents to carry out the above-described assays.

Specifically, the invention provides a compartmentalized kit to receive, in close confinement, one or more containers which comprises: (a) a first container comprising one of the antibodies, or one of the DFs of the present invention; and (b) one or more other containers comprising one or more of the following: wash reagents, reagents capable of detecting presence of bound antibodies or hybridized DFs.

Using the isolated proteins of the present invention, the present invention further provides methods of obtaining and identifying agents capable of binding to a polypeptide or protein encoded by one of the ORFs of the present invention. Specifically, such agents include, as further described below, antibodies, peptides, carbohydrates, pharmaceutical agents and the like. Such methods comprise steps of: (a) contacting an agent with an isolated protein encoded by one of the ORFs of the present invention; and (b) determining whether the agent binds to said protein.

The present genomic sequences of *Streptococcus pneumoniae* will be of great value to all laboratories working with this organism and for a variety of commercial purposes. Many fragments of the *Streptococcus pneumoniae* genome will be immediately identified by similarity searches against GenBank or protein databases and will be of immediate value to *Streptococcus pneumoniae* researchers

and for immediate commercial value for the production of proteins or to control gene expression.

The methodology and technology for elucidating extensive genomic sequences of bacterial and other genomes has and will greatly enhance the ability to analyze and understand chromosomal organization. In particular, sequenced contigs and genomes will provide the models for developing tools for the analysis of chromosome structure and function, including the ability to identify genes within large segments of genomic DNA, the structure, position, and spacing of regulatory elements, the identification of genes with potential industrial applications, and the ability to do comparative genomic and molecular phylogeny.

DESCRIPTION OF THE FIGURES

FIGURE 1 is a block diagram of a computer system (102) that can be used to implement computer-based systems of present invention.

FIGURE 2 is a schematic diagram depicting the data flow and computer programs used to collect, assemble, edit and annotate the contigs of the *Streptococcus pneumoniae* genome of the present invention. Both Macintosh and Unix platforms are used to handle the AB 373 and 377 sequence data files, largely as described in Kerlavage *et al.*, *Proceedings of the Twenty-Sixth Annual Hawaii International Conference on System Sciences*, 585, IEEE Computer Society Press, Washington D.C. (1993). Factura (AB) is a Macintosh program designed for automatic vector sequence removal and end-trimming of sequence files. The program Loadis runs on a Macintosh platform and parses the feature data extracted from the sequence files by Factura to the Unix based *Streptococcus pneumoniae* relational database. Assembly of contigs (and whole genome sequences) is accomplished by retrieving a specific set of sequence files and their associated features using Extrseq, a Unix utility for retrieving sequences from an SQL database. The resulting sequence file is processed by seq_filter to trim portions of the sequences with more than 2% ambiguous nucleotides. The sequence files were assembled using TIGR Assembler, an assembly engine designed at The Institute for Genomic Research (TIGR) for rapid and accurate assembly of thousands of sequence fragments. The collection of contigs generated by the assembly step is loaded into the database with the lassie program. Identification of open reading

GTTTCGATTG GAGTTGTTGT TGGAAATTGT GTTTTTTCTA CAACGTTAAA GTTTTCATCA 7620

CCGACAGCAC AGACAAACTT TGTACCGCCC GCTTCCAAGC TTCCATATAA TTTTGTCATG 7680

ATAAACCTCT TGTTTTTATT TTCTTTATTA TAGCATACTT CGAAAGTCTA AATGTCTCTA 7740

TTTTTTAGAT TTTCCTCTGT AAATCTTACT ATCTAATAAA AACGAACAAA CATGTCATTT 7800

GTTCGTTTTC ACATTAGAGA GGATTGATTA GATTTTCACT TCGATCACAG CATCCCCCTT 7860

AGCAACTGAA CCTGTTGCGA CTGGAGCTAC TGAAGCGTAG TCACCTGTAT TTGTAACGAT 7920

AACCATTGTT GTATCATCAA GTCCAGCTGC AGCGATTTTG TTTGAGTCAA ATGTTCCAAG 7980

AACATCGCCA GCTTTCACCT TATTACCTTG AGCAACTTTT GTTTCAAAAC CGTCACCGTT 8040

CATAGATACA GTATCAATAC CAACATGAAT CAAAACTTCA GCACCATTTC TTGTTTTCAA 8100

ACCAAAAGCG TGCCCTGTTG GAAAGGCAAT TGAAACTTCA GCATCAGCTG GTGCATAGAC 8160

CACGCCTTGG CTTGGTTTCA CAACGATACC TTGTCCCATA GCTCCACTTG AGAAGACTGG 8220

GTCATTGACA TCAGCAAGAG CGACAACATC ACCGACGATA GGAGTTACAA GTGTTTCATT 8280

TTGAAGAGCT GCTGGCGCAA CTTCTTCTTT TTCTTCAGCC ACTTCAGCTC GTTTTGCACC 8340

TGCAGTTGCG TCTACTTCAT CTTCGTAACC AAACATGTAA GTAAGAGCAA AACCAAGGGC 8400

AAATGATACA GCTACCATAA GAAGGTATTG TGGAAGTTGT CCGTTACCAA CATAAAGCAT 8460

TGTACCAGGG ATGATGGTGA TACCATTACC AGTACCAGCA AGTCCAAGGA TAGAAGCCAA 8520

TCCACCACCG ATTGCACCAG CAATCAATGA AAGGAAGAAT GGTTTACGGA AGCGCAAGTT 8580

CACCCCGAAG ATAGCAGGCT CTGTAATACC TAGGAAGGCA GAAAGAGCAG CCGGGAAAGC 8640

AAGTGTTTTC AGTTTTGGAT TTTTTGTTTT AACACCAACC GCAACAGTAG CAGCACCTTG 8700

AGCTGTCATA GCAGCTGTGA TGATAGCGTT GAATGGGTTA GCATGGTCAG CAGCAAGTAA 8760

TTGCACTTCA AGCAAGTTGA AGATGTGGTG CACACCTGAC ACGACGATCA ATTGGTGAAC 8820

CCCACCAATC AAGAAACCAC CAAGACCAAA TGGCATGCTA AGAATCGCTT TTGTAGCAAT 8880

AAGGATGTAG TTTTCAACAA CGTGGAAAAC TGGTCCAATG ACAAAGAGTC CAAGGATAGA 8940

CATGACCAAA AGTGTCACGA ATGGTGTTAC CAAGAGGTCA ATGACATCTG GAACAACTTG 9000

CGGACAGCTT TTTCAAATTT AGCTCCGACA ACCCCGATGA TGAAGGCTGG AAGAACGGAA 9060

CCTTGCAAAC CAACAACAGG GATGAAACCA AAGAAGTTCA TCGCTGTTAC TTCACCACCT 9120

TGAGCAACTG CCCAAGCGTT TGGAAGTGAG CCAGAGACAA GCATCATACC AAGAACGATA 9180

CCAACGGCAG GATTTCCACC AAATACACGG AAGGTTGACC ACACAACCAA ACCTGGCAAG 9240

ATCATGAAGG CTGTATCTGT CAAGATTTGT GTGTAAGTTG CAAAGTCACC TGGAAGTGGC 9300

What Is Claimed Is:

1. Computer readable medium having recorded thereon the nucleotide sequence depicted in SEQ ID NOS:1-391, a representative fragment thereof or a nucleotide sequence at least 95% identical to a nucleotide sequence depicted in SEQ ID NOS:1-391.

2. Computer readable medium having recorded thereon any one of the fragments of SEQ ID NOS:1-391 depicted in Tables 2 and 3 or a degenerate variant thereof.

3. The computer readable medium of claim 1, wherein said medium is selected from the group consisting of a floppy disc, a hard disc, random access memory (RAM), read only memory (ROM), and CD-ROM.

4. The computer readable medium of claim 3, wherein said medium is selected from the group consisting of a floppy disc, a hard disc, random access memory (RAM), read only memory (ROM), and CD-ROM.

5. A computer-based system for identifying fragments of the *Streptococcus pneumoniae* genome of commercial importance comprising the following elements:

a) a data storage means comprising the nucleotide sequence of SEQ ID NOS:1-391, a representative fragment thereof, or a nucleotide sequence at least 95% identical to a nucleotide sequence of SEQ ID NOS:1-391;

b) search means for comparing a target sequence to the nucleotide sequence of the data storage means of step (a) to identify homologous sequence(s), and

c) retrieval means for obtaining said homologous sequence(s) of step (b).

6. A method for identifying commercially important nucleic acid fragments of the *Streptococcus pneumoniae* genome comprising the step of comparing a database comprising the nucleotide sequences depicted in SEQ ID NOS:1-391, a representative fragment thereof, or a nucleotide sequence at least 95% identical to a nucleotide sequence of SEQ ID NOS:1-391 with a target sequence to obtain a nucleic acid molecule comprised of a complementary nucleotide sequence to said target sequence, wherein said target sequence is not randomly selected.

7. A method for identifying an expression modulating fragment of *Streptococcus pneumoniae* genome comprising the step of comparing a database comprising the nucleotide sequences depicted in SEQ ID NOS:1-391, a representative fragment thereof, or a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID NOS:1-391 with a target sequence to obtain a nucleic acid molecule comprised of a complementary nucleotide sequence to said target sequence, wherein said target sequence comprises sequences known to regulate gene expression.

8. An isolated protein-encoding nucleic acid fragment of the *Streptococcus pneumoniae* genome, wherein said fragment consists of the nucleotide sequence of any one of the fragments of SEQ ID NOS:1-391 depicted in Tables 2 and 3, or a degenerate variant thereof.

9. A vector comprising any one of the fragments of the *Streptococcus pneumoniae* genome SEQ ID NOS:1-391 depicted in Tables 2 and 3 or a degenerate variant thereof.

10. An isolated fragment of the *Streptococcus pneumoniae* genome, wherein said fragment modulates the expression of an operably linked open reading frame, wherein said fragment consists of the nucleotide sequence from about 10 to 200 bases in length which is 5' to any one of the open reading frames depicted in Tables 2 and 3 or a degenerate variant thereof.

11. A vector comprising any one of the fragments of the *Streptococcus pneumoniae* genome of claim 8.

12. An organism which has been altered to contain any one of the fragments of the *Streptococcus pneumoniae* genome of claim 8.

13. An organism which has been altered to contain any one of the fragments of the *Streptococcus pneumoniae* genome of claim 10.

14. A method for regulating the expression of a nucleic acid molecule comprising the step of covalently attaching to said nucleic acid molecule a nucleic acid molecule consisting of the nucleotide sequence from about 10 to 100 bases 5' to any one of the fragments of the *Streptococcus pneumoniae* genome depicted in SEQ ID NOS:1-391 and Tables 2 and 3 or a degenerate variant thereof.

15. An isolated nucleic acid molecule encoding a homolog of any of the fragments of the *Streptococcus pneumoniae* genome of SEQ ID NOS:1-391 and Tables 2 and 3, wherein said nucleic acid molecule is produced by a process comprising steps of:

a) screening a genomic DNA library using as a probe a target sequence defined by any of SEQ ID NOS:1-391 and Tables 2 and 3, including fragments thereof;

b) identifying members of said library which contain sequences that hybridize to said target sequence; and

c) isolating the nucleic acid molecules from said members identified in step (b).

16. An isolated DNA molecule encoding a homolog of any one of the fragments of the *Streptococcus pneumoniae* genome of SEQ ID NOS:1-391 and Tables 2 and 3, wherein said nucleic acid molecule is produced a process comprising steps of:

a) isolating mRNA, DNA, or cDNA produced from an organism;

b) amplifying nucleic acid molecules whose nucleotide sequence is homologous to amplification primers derived from said fragment of said *Streptococcus pneumoniae* genome to prime said amplification;

c) isolating said amplified sequences produced in step (b).

17. An isolated polypeptide encoded by any of the fragments of the *Streptococcus pneumoniae* genome of SEQ ID NOS:1-391 and depicted in Table 2 and 3 or by a degenerate variant of said fragments.

18. An isolated polynucleotide molecule encoding any one of the polypeptides of claim 17.

19. An antibody which selectively binds to any one of the polypeptides of claim 17.

20. A method for producing a polypeptide in a host cell comprising the steps of:

a) incubating a host containing a heterologous nucleic acid molecule whose nucleotide sequence consists of any one of the fragments of the *Streptococcus pneumoniae* genome of SEQ ID NOS:1-391 and depicted in Tables 2 and 3, under conditions where said heterologous nucleic acid molecule is expressed to produce said protein, and

b) isolating said protein.